

Reshaping Waddington's developmental landscape

Imagine 2004, prior to the advent of induced pluripotent stem cells (iPS cells) and the realization that cellular identity is a tabula rasa that can be rewritten at will. It was widely accepted that once terminally differentiated cells were born, their identity – be it a brain, bone or blood cell – was fixed and immutable. This view was influenced by Waddington's landscape model from his 1940 monograph, *Organisers and Genes*, which depicted cellular differentiation as an irreversible, one-way journey down a slope. However, in 1940, Haldane presciently presaged the decades to come; "Like other symbols in biology, I expect that this one will prove valuable for a time but may later be rather misleading."

In the 1980s, Weintraub, Graf and others challenged this dogma by demonstrating that the forced expression of a single transcription factor could reprogramme the identity of cultured cells. Yet, these early studies encountered resistance, with some even dismissing reprogramming as an experimental artefact. This raised a critical question: are the identities of freshly isolated, differentiated cells similarly malleable?

A pivotal answer came from a 2004 study led by Thomas Graf, which was one of the first to show that differentiated adult cells could be reprogrammed to adopt starkly different identities by the forced expression of a single transcription factor. This discovery represented a watershed moment in our understanding of cellular stability and reprogramming. It also cemented the foundation for the subsequent reprogramming of fibroblasts into iPS cells by Takahashi and Yamanaka in 2006.

Xie et al. freshly isolated B cells from the bone marrow or spleen of adult mice. Left to their own devices, these B cells stably maintained their identity in culture. However, the forced overexpression of a single transcription factor, CEBPA, reprogrammed these B cells into macrophages. Multiple aspects of this reprogramming event were particularly salient.

First, it entailed a dramatic alteration in cell identity and function: B cells secrete antibodies, whereas macrophages (literally translated to 'big eaters' from Greek)

physically consume invading pathogens. Indeed, the reprogrammed macrophages engulfed bacteria, as subsequent work revealed. Second, reprogramming was extraordinarily fast and efficient: >80% of B cells became macrophages within just 4 days. Third, B cells were unequivocally reprogrammed into macrophages. The genomes of the reprogrammed macrophages harboured unambiguous rearrangements in antibody-encoding genes, elegantly confirming the B cell ancestry of the newly inaugurated macrophages. Fourth, this reprogramming was not limited to cell culture; CEBPA-expressing B cells injected into mice also underwent reprogramming in vivo.

Reprogramming constitutes an intense battle between opposing forces. Within B cells, the transcription factor PAX5 is dominant and continuously represses macrophage identity. When overexpressed, CEBPA usurps control from PAX5. CEBPA achieves two remarkable objectives: silencing highly expressed B cell identity genes, while simultaneously activating macrophage genes buried in silent chromatin. Subsequent work showed that CEBPA dominates over PAX5 by silencing *PAX5* expression, allowing it to seize genomic control.

A fascinating and underappreciated discovery by Xie et al. is the necessity of cooperation between exogenous CEBPA and the endogenous transcription factor PU.1. PU.1 is a general immune cell transcription factor expressed by both B cells and macrophages. While overwhelming attention in the reprogramming field is devoted to the exogenously overexpressed transcription factors, this study revealed that exogenous and endogenous transcription factors must cooperate to reprogramme cells. When overexpressed, CEBPA interacts with PU.1 in B cells to cooperatively activate a suite of macrophage genes. CEBPA fails to reprogramme PU.1-deficient B cells, underscoring how exogenously and endogenously expressed transcription factors must liaise to reprogramme cells. Later studies showed that CEBPA fails to reprogramme fibroblasts, which lack PU.1

expression, unless PU.1 is overexpressed in addition to CEBPA.

Thus, the developmental relatedness of B cells and macrophages – manifested by their shared PU.1 expression – is key to enable CEBPA to achieve B cell-to-macrophage reprogramming. The roles of endogenous transcription factors in reprogramming are largely overlooked, but probably explain various reports where the same set of exogenous transcription factors can reprogramme some, but not all, cell types.

"exogenous and endogenous transcription factors must cooperate to reprogramme cells"

Notably, two decades later, the Xie et al. study has endured the test of time. Numerous laboratories around the world have reprogrammed B cells into macrophages using CEBPA. De novo macrophage production has afforded new insights into reprogramming mechanisms and macrophage-mediated inflammation and has even been used to treat cancer. By demonstrating that B cells could be reprogrammed into macrophages with high efficiency and speed, this study marked a pivotal revision of Waddington's developmental landscape, setting the stage for our modern understanding that cellular identity is far more malleable than previously imagined.

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Competing interests

The authors declare no competing interests.

Original article: Xie, H. et al. Stepwise reprogramming of B cells into macrophages. *Cell* **117**, 663–676 (2004)

Related article: Graf, T. Historical origins of transdifferentiation and reprogramming. *Cell Stem Cell* **9**, 504–516 (2011)